Meat Mutagens and Risk of Distal Colon Adenoma in a Cohort of U.S. Men

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Abstract

Cooking meats at high temperatures and for long duration produces heterocyclic amines and other mutagens. These meat-derived mutagenic compounds have been hypothesized to increase risk of colorectal neoplasia, but prospective data are unavailable. We examined the association between intakes of the heterocyclic amines 2-amino-3,8-dimethylimidazo[4,5,-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,4,8-trimethylimidazo[4,5,-*f*]quinoxaline (DiMeIQx), and meat-derived mutagenicity (MDM) and risk of distal colon adenoma using a cooking method questionnaire administered in 1996 in the Health Professionals Follow-up Study cohort. Between 1996 and 2002, 581 distal colon adenoma cases were identified. Higher intake of MDM was marginally associated with increased risk of distal adenoma [fourth versus lowest quintile: odds ratio (OR), 1.39; 95% confidence interval (95% CI), 1.05-1.84; highest versus lowest quintile: OR, 1.29; 95% CI, 0.97-1.72; P_{trend} = 0.08]. Adjusting for total red meat or processed meat intake did not explain those associations. Our data also suggested a positive association between higher MeIQx (highest versus lowest quintile: OR, 1.28; 95% CI, 0.95-1.71; P_{trend} = 0.22) and risk of adenoma, but this association was attenuated after adjusting for processed meat intake. DiMeIQx and PhIP did not seem to be associated with risk of adenoma. In conclusion, higher consumption of mutagens from meats cooked at higher temperature and longer duration may be associated with higher risk of distal colon adenoma independent of overall meat intake. Because mutagens other than heterocyclic amines also contribute to MDM, our results suggest that mutagens other than heterocyclic amines in cooked meats may also play a role in increasing the risk of distal adenoma. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1120–5)

Introduction

In many epidemiologic studies, meat consumption, particularly red meat, has been associated with higher risk of colorectal cancers or adenomas (1-4). Meat may be involved in colorectal carcinogenesis via several biological mechanisms. One possible mechanism involves the formation of heterocyclic amines, which are produced when meats are cooked at high temperatures and for long duration (5). Although heterocyclic amines are mutagenic in animal and *in vitro* studies and are carcinogenic in animal studies (5), results from epidemiologic studies of cooking methods and risk of colorectal cancers or their precursors colorectal adenoma (6, 7) have not been consistent possibly due in part to confounding by other components of meat and possible misclassification of intake (2, 8). Heterocyclic amine concentrations in cooked meats increase with increasing temperature and duration of cooking but also depend on cooking methods and type of meat (5, 9). Therefore, studying cooking methods alone as a marker for heterocyclic amine intake may not adequately assess heterocyclic amine exposure (8).

Recently, Sinha et al. developed a method to estimate heterocyclic amine in specific types of meat using proxies for high-temperature cooking and time. Heterocyclic amine intake can be calculated based on these measurements and dietary intake of specific types of meat prepared with different cooking methods and at various doneness levels (5, 10-13). In addition to heterocyclic amine measurements, the database developed by Sinha et al. (http://www.charred.cancer.gov) also contains data on meat-derived mutagenicity (MDM). MDM measures overall mutagenic activity in cooked meat (14, 15) and also increases with higher cooking temperature and duration (http://www.charred.cancer.gov). Thus, in addition to the mutagenic activity due to heterocyclic amines, MDM incorporates mutagenic activities from other compounds found in cooked meats (15). Epidemiologic data on heterocyclic amine or MDM intake and risk of colon or colorectal cancers or adenomas are sparse. Three U.S. case-control studies have found that higher intakes of certain heterocyclic amines may increase risk of colon or colorectal cancers or adenomas (8, 15, 16). One case-control study from Sweden did not observe an overall association between heterocyclic amine intake and risk of colon cancer (17) but found some suggestion of an increased colon cancer risk at the very high end of total heterocyclic amine intake (>1,900 ng/d; ref. 17). Only one case-control study has examined the association between heterocyclic amine intake and MDM and risk of colorectal adenoma (15). In that study, higher intake of 2-amino-3,4,8-trimethylimidazo[4,5,-*f*]quinoxaline (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and MDM were associated with higher risk of colorectal adenoma (15). However, assessing exposure after diagnosis of the disease raises the concern that bias in the recall of dietary habits may explain reported associations.

To address this concern, we examined the associations between heterocyclic amine and MDM intake and meat intake in a prospective manner using data from a cooking method questionnaire and food frequency questionnaires (FFQ) administered in the Health Professionals Follow-up Study (HPFS) cohort.

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Materials and Methods

Study Population. The HPFS cohort was formed in 1986 when 51,129 male U.S. health professionals returned a baseline questionnaire on medical history and lifestyle factors. A 131-item FFQ was included with the baseline questionnaire (18). Follow-up questionnaires on medical history and lifestyle factors were mailed every 2 years and additional FFQs were included every 4 years. In 1996, a section requesting information on typical cooking methods was included in the regular biennial follow-up questionnaire. This study was approved by the Human Subjects Committee of the Harvard School of Public Health.

We excluded men with history of ulcerative colitis or cancer (except for nonmelanoma skin cancer and organ-confined prostate cancer) or colorectal polyps before 1996, men who did not respond to the 1996 questionnaires, and those who had left the entire cooking method section blank, did report doneness but not frequency of meat intake of at least one cooked meat item, or did not have information on bacon intake from the 1994 FFQ (see below). Colorectal adenomas, which in most cases are asymptomatic, are mainly detected during routine screening endoscopies or other endoscopies done for adenoma unrelated symptoms. Therefore, after exclusions, our analysis was restricted to 14,032 men who had a large bowel endoscopy between 1996 (the year the cooking method questionnaire was administered) and 2002.

Ascertainment of Colon Adenoma Cases. For men who reported a diagnosis of colorectal polyp on their biennial follow-up questionnaire, we mailed a consent form with a request to review their medical records. These medical records were reviewed by study investigators who extracted information on location, histology, and size of the polyp. All adenoma cases for analysis had to be confirmed by pathology reports. Because we do not know whether a participant who underwent endoscopy had a sigmoidoscopy alone (a sigmoidoscopy only examines the distal part of the colorectum), only distal colon adenomas were included in our analysis. We did not include rectal adenomas because the etiology of colon and rectal cancer may differ (19) and numbers were insufficient to consider rectal cases separately.

Of the 14,032 men who had a large bowel endoscopy between 1996 and 2002, a total of 581 distal colon adenomas were diagnosed between 1996 and 2002. The remaining 13, 451 participants, including those participants who were diagnosed with proximal and/or rectal adenoma, were treated as noncases.

Charred Database. The Charred Database developed by Sinha et al. is an online database that contains data on heterocyclic amine and MDM (http://www.charred.cancer.gov). 2-Amino-3,8-dimethylimidazo[4,5,-*f*]quinoxaline (MeIQx), DiMeIQx, and PhIP were measured in meat samples applying a method described by Gross and Gruter (20). The mutagenic activity of the meat sample extracts were determined by applying the Ames/*Salmonella* test (14, 21).

Assessment of Diet, Meat, and Heterocyclic Amine Intake. We computed nutrient intake by multiplying the nutrient content of foods with the reported frequency of intake of each food from the 1986, 1990, 1994, and 1998 FFQs and applied the residuals method to calculate energy-adjusted nutrient intakes (22). We reported the validation and reproducibility of the FFQs administered in this cohort in previous publications (23-25). Cumulative updated nutrient intake was computed by averaging the nutrient intakes from all available questionnaires (1986, 1990, 1994, and 1998) before the beginning of each 2-year follow-up interval (22). We used cumulative average intake to enhance our estimate of long-term dietary intake (22).

The cooking method questions in the 1996 questionnaire were based on results from a previous pilot study, which determined the group of cooking method questions that would best predict heterocyclic amine intake in this specific cohort (26) and included questions on frequency of intake (never, <1/mo, 1/mo, 2-3/mo, 1/wk, 2-3/wk, and ≥4/wk) and outside appearance (depending on type of meat: lightly browned, medium browned, well browned, and blackened/charred) of meats and fish. The items included pan-fried, broiled, and grilled chicken, broiled fish, roast beef, pan-fried steak, grilled or barbecued steak, and homemade beef gravy. We also included questions on whether the chicken was cooked with skin on and whether the skin was eaten. Our previous pilot study found limited variation in the reported doneness of cooking bacon (26). Therefore, frequency of fried bacon intake was based on bacon intake from the 1994 FFQ and assumed to be prepared at higher doneness levels (medium browned; ref. 26). We estimated intake of each individual heterocyclic amine (i.e., MeIQx, PhIP, and DiMeIQx) and MDM by multiplying the frequency of cooked meat intake from the 1996 cooking questionnaire with measured individual heterocyclic amine (ng/g meat) or MDM levels (revertant colonies/g meat) and standard (medium) portion size from the Charred Database. We also examined associations between the predicted heterocyclic amine mutagenicity (PHM; i.e., the mutagenicity that can be attributed to all measured heterocyclic amines) and adenoma. PHM was calculated by multiplying the amount of heterocyclic amines measured in the sample and multiplying them with the mutagenic capacity for that compound and then summing up the mutagenicity for all heterocyclic amines (http://www.charred.cancer.gov).

Because the cooking questionnaire did not assess frequency of consumption of specific meats (i.e., total meat intake regardless of cooking method), we computed total intake of specific meats using information from the 1986 to 1994 FFQs (cumulative average intake, see above). Among the study participants, the vast majority (88%) completed all questions on frequency of intake of meat prepared with different cooking methods. For missing data among participants who had reported frequency of intake but left the doneness section blank, we imputed the mode for doneness (lightly brown for broiled fish and medium browned for all other meat items). For participants who had reported nonzero intake of chicken but did not report whether the chicken was cooked or eaten with skin, we assumed that they did not cook or eat chicken skin, because the majority of participants who had reported nonzero intake of chicken did not cook (51%) or eat chicken skin (76%).

Statistical Analysis. We used multivariable logistic regression models and calculated odds ratios (OR) to assess associations between dietary variables and adenoma risk. We included known and suspected risk factors for colorectal adenoma in our final models (shown in footnotes in tables). All dietary variables, age, physical activity, and smoking status, were updated up to the 1998 questionnaire or the year of the questionnaire before the most recent endoscopy (for cases, this would be the endoscopy that led to the diagnosis of adenoma; for controls, this would be the year of the most recent reported endoscopy), whichever came first. In addition, we assessed possible confounding from other dietary factors by adding each other dietary factor separately to the final multivariable models. We computed trend tests by adding a continuous variable using the median for each quintile of the exposure (i.e., heterocyclic amine, MDM, and meat intake) to the multivariable models.

Results

Table 1 shows the baseline characteristics in the HPFS by quintiles of heterocyclic amine intake and MDM. Participants

Table 1. Baseline characteristics in the HPFS study population by lowest and highest quintiles of heterocyclic amine and MDM intake

	PhIP (ng/d)		MeIQx (ng/d)		DiMeIQx (ng/d)		MDM (revertant colonies/d)	
	Q1 (14.4)	Q5 (220.4)	Q1 (1.5)	Q5 (35.0)	Q1 (0)	Q5 (4.0)	Q1 (711)	Q5 (8,125)
Mean age (y)	65.0	59.7	63.6	62.3	64.0	60.1	64.2	60.9
Race (%)*								
Southern European	19.4	21.7	22.4	18.0	22.2	21.0	21.5	19.9
Northern European	70.2	67.3	65.7	71.8	67.4	69.7	67.4	70.1
Others	3.8	5.0	4.5	3.6	4.0	3.4	4.3	4.1
Current smokers (%)	3.0	4.7	1.3	6.7	2.7	4.9	2.1	5.4
Mean pack-years smoking	9.8	12.6	9.2	13.6	10.6	12.0	9.7	12.7
Family history of colorectal cancer (%)	17.2	17.1	17.5	16.6	16.5	15.9	16.9	16.4
Aspirin use (>2/wk; %)	52.8	55.3	55.3	54.8	54.7	57.2	53.9	56.9
Mean height (inches)	70.1	70.2	69.9	70.5	70.1	70.3	70.0	70.3
Body mass index (kg/m^2)	25.0	25.9	24.8	26.1	25.4	25.8	25.0	25.9
Physical activity (MET)	34.7	34.5	36.7	32.4	34.4	34.7	34.6	33.7
Mean daily intake† (94 FFQ)								
Calories (kcal)	1,864	2,128	1,796	2,265	1,910	2,108	1,827	2,173
Total fat (g)	60.2	67.4	56.0	72.6	62.3	65.8	60.1	67.1
Animal protein (g)	54.3	63.8	54.9	62.9	58.9	62.1	53.9	63.6
Vegetable protein (g)	30.0	26.2	31.2	87.8	28.5	27.0	30.1	26.4
Total iron (mg)	22.5	20.0	22.6	19.5	21.9	20.3	22.2	20.4
Heme iron (mg)	0.9	1.3	0.9	1.3	1.1	1.2	0.92	1.3
Folate (μg)	583	529	612	485	567	527	584	525
Methionine (g)	1.9	2.1	2.0	2.1	2.0	2.1	1.9	2.1
Alcohol (g)	9.1	13.8	8.7	12.7	10.5	12.3	9.5	12.4
Vitamin C (mg)	565	465	613	392	528	452	568	445
Total carotene (IU)	15,027	12,463	16,488	10,794	14,279	12,646	15,054	12,282
Vitamin D (IU)	493	444	522	407	488	436	498	440
Calcium (mg)	1,012	860	1,013	867	967	896	995	884
Total fiber (g)	25.8	21.9	27.4	20.3	24.4	22.5	25.8	22.0
Red meat (servings/d)	0.35	0.65	0.24	0.84	0.43	0.62	0.31	0.69
Processed meat (servings/d)	0.17	0.31	0.10	0.46	0.21	0.28	0.15	0.34
Chicken + turkey (servings/d)	0.33	0.48	0.38	0.39	0.39	0.44	0.33	0.47

NOTE: Standardized for age in 1996. Q1 is lowest quintile and Q5 is highest quintile. Numbers in parentheses are median value in each quintile (ng/d for heterocyclic amines and revertant colonies/d for MDM).
Abbreviation: MET, metabolic equivalent-hours/wk.
*Due to missing values, percentages for race do not add up to 100%.
†Mean daily intakes of nutrients are energy adjusted.

in the highest quintile of PhIP, MeIQx, and DiMeIQx intake and MDM had a slightly higher number of pack-years of smoking and were more likely to be current smokers compared with those in the lowest quintile of intake. In addition, participants in the highest quintile of MeIQx intake seemed to be less physically active than those in the lowest quintile. Higher intake of heterocyclic amine and higher MDM were associated with higher intake of total energy and certain nutrients, including total fat, animal protein, heme iron, and alcohol, but with lower intake of total iron, vitamin C, total carotene, vitamin D, calcium, and fiber.

Intakes of MeIQx, PhIP, and DiMeIQx were moderately correlated with each other (MeIQx versus PhIP: $r = 0.54$, MeIQx versus DiMeIQx: $r = 0.45$, PhIP versus DiMeIQx: $r = 0.47$). Heterocyclic amines and MDM were also correlated (DiMeIQx versus MDM: $r = 0.41$, PhIP versus MDM: $r = 0.68$, MeIQx versus MDM: $r = 0.70$). Table 2 shows mean intake and percentage contribution of heterocyclic amine intake and MDM by meats prepared with different cooking methods. Grilled chicken, grilled steak, and broiled chicken contributed most to total PhIP intake, whereas pan-fried hamburgers and grilled steak contributed most to total MeIQx intake. The most important contributors to total DiMeIQx intake were grilled chicken and pan-fried hamburgers. Broiled chicken, grilled chicken, and pan-fried hamburgers contributed most to MDM.

Table 3 shows the ORs and 95% confidence intervals (95% CI) of distal adenoma by quintiles of heterocyclic amine intake and MDM before and after adjusting for cumulative average total red meat and processed meat intake. Higher intake of MDM was marginally associated with increased risk of adenoma. Adjusting for total red meat or processed meat intake attenuated associations only slightly. Our data also suggested a positive association between higher MeIQx and risk of adenoma, but this association was attenuated after

Table 2. Mean intake of heterocyclic amine and MDM by meat type, HPFS

	PhIP		MeIQx		DiMeIQx		MDM	
	Mean (SD), ng/d	%	Mean (SD), ng/d	%	Mean (SD), ng/d	%	Mean (SD), revertant colonies/d	%
All sources	103 (110)		15.4 (18.0)		1.4 (2.4)		3,718 (3,272)	
Pan-fried chicken	8.9 (23.9)	8.6	1.05 (2.3)	6.8	0.05 (0.21)	3.6	457 (1,047)	12.3
Broiled chicken	23.6 (44.2)	22.9	0.38 (0.88)	2.5	0.01 (0.17)	0.7	982 (1,695)	26.4
Grilled chicken	43.5 (71.2)	42.2	1.2 (2.5)	7.8	0.88 (2.2)	62.9	850 (1,277)	22.9
Pan-fried hamburger	0.06 (1.5)	0.1	6.5 (12.3)	42.2	0.32 (0.72)	22.9	647 (1,274)	17.4
Grilled steak	25.9 (45.7)	25.1	3.2 (4.9)	20.8	0	N/A	535 (1,120)	14.4
Fried bacon	0.76 (1.7)	0.7	1.8 (4.2)	11.7	0	N/A	110 (251)	3.0
Homemade beef gravy	0.43 (2.7)	0.4	1.3 (4.7)	8.4	0.11 (0.71)	7.9	136 (538)	3.7

Table 3. ORs (95% CIs) of distal colon adenoma by quintiles of heterocyclic amine and MDM intake before and after adjusting for red and processed meat intake, HPFS

	Heterocyclic amine intake (ng/d)/mutagenicity (revertant colonies/d)					P_{trend}
	1 (Low)	2	3	4	5	
Total MeIQx intake (ng/d), median	1.5	5.3	9.9	17.3	35.0	
Cases/controls	88/2,720	113/2,693	119/2,688	124/2,681	137/2,669	
Multivariable*	1.00	1.18 (0.88-1.57)	1.22 (0.91-1.63)	1.19 (0.89-1.59)	1.28 (0.95-1.71)	0.22
Multivariable (+red meat)	1.00	1.15 (0.86-1.54)	1.17 (0.87-1.58)	1.15 (0.84-1.56)	1.23 (0.90-1.68)	0.34
Multivariable (+processed meat)	1.00	1.12 (0.84-1.50)	1.13 (0.84-1.52)	1.08 (0.80-1.46)	1.14 (0.83-1.55)	0.66
Total PhIP intake (ng/d), median	14.4	39.1	70.6	117.4	220.4	
Cases/controls	108/2,696	110/2,700	108/2,699	122/2,683	133/2,673	
Multivariable*	1.00	0.97 (0.74-1.28)	0.93 (0.71-1.23)	1.05 (0.80-1.38)	1.11 (0.85-1.46)	0.25
Multivariable (+red meat)	1.00	0.95 (0.72-1.26)	0.91 (0.69-1.20)	1.03 (0.78-1.35)	1.09 (0.83-1.44)	0.29
Multivariable (+processed meat)	1.00	0.95 (0.72-1.25)	0.89 (0.68-1.18)	1.01 (0.77-1.33)	1.08 (0.82-1.42)	0.32
Total DiMeIQx intake (ng/d), median	0	0.2	0.5	1.2	4.0	
Cases/controls	183/4,372	39/1,266	95/2,222	129/2,873	135/2,718	
Multivariable*	1.00	0.72 (0.51-1.03)	0.97 (0.75-1.25)	0.99 (0.79-1.26)	1.08 (0.86-1.37)	0.22
Multivariable (+red meat)	1.00	0.71 (0.50-1.02)	0.96 (0.74-1.24)	0.97 (0.77-1.23)	1.07 (0.85-1.36)	0.25
Multivariable (+processed meat)	1.00	0.70 (0.49-1.00)	0.94 (0.73-1.22)	0.96 (0.75-1.21)	1.06 (0.84-1.35)	0.25
MDM (revertant colonies/d), median	711	1,736	2,831	4,347	8,125	
Cases/controls	90/2,718	100/2,705	127/2,676	135/2,672	129/2,680	
Multivariable*	1.00	1.05 (0.78-1.41)	1.32 (1.00-1.75)	1.39 (1.05-1.84)	1.29 (0.97-1.72)	0.08
Multivariable (+red meat)	1.00	1.03 (0.77-1.39)	1.30 (0.98-1.73)	1.36 (1.03-1.81)	1.26 (0.94-1.69)	0.10
Multivariable (+processed meat)	1.00	1.02 (0.76-1.37)	1.27 (0.96-1.69)	1.33 (1.00-1.77)	1.23 (0.92-1.65)	0.13

*Models adjusted for age (in 5-year categories), family history of colorectal cancer (first-degree relative), reason for endoscopy (screening versus others), negative endoscopy before 1996 (yes versus no), physical activity (metabolic equivalent-hours/wk in quintiles), smoking status (never smokers, past smokers who quit ≤10 years ago, past smokers who quit >10 years ago, current smokers), race, aspirin use (<2/wk versus ≥2/wk), total energy intake (in quintiles), and calcium and folate intake (in quintiles).

adjusting for processed meat intake. DiMeIQx and PhIP did not seem to be associated with risk of adenoma.

We investigated associations between cumulative average meat intake regardless of cooking method and risk of adenoma before and after adjusting for MeIQx and MDM intake (Table 4). We found a positive association between higher intake of processed meat [defined as the sum of the following meat items on the FFQs: processed meats (sausage, salami, bologna, etc.), bacon, and hotdogs] and risk of adenoma. Chicken and turkey intake was not associated with adenoma risk.

When MDM and all three heterocyclic amines were modeled simultaneously in one model, positive associations between MDM and adenoma were not altered appreciably

Table 4. ORs (95% CIs) of distal colon adenoma by quintiles of meat intake before and after adjusting for MDM and MeIQx, HPFS

	Categories of meat intake					P_{trend}
	1 (Low)	2	3	4	5	
Total red meat (servings/wk), median	1.1	2.3	3.4	4.8	7.2	
Cases/controls	96/3,006	97/2,285	128/2,779	124/2,702	136/2,679	
Multivariable*	1.00	1.16 (0.86-1.55)	1.24 (0.93-1.64)	1.19 (0.88-1.59)	1.18 (0.87-1.62)	0.43
Multivariable (+MDM)	1.00	1.12 (0.84-1.51)	1.19 (0.89-1.58)	1.12 (0.83-1.51)	1.10 (0.80-1.52)	0.75
Multivariable (+MeIQx)	1.00	1.12 (0.83-1.51)	1.17 (0.88-1.58)	1.11 (0.81-1.52)	1.10 (0.79-1.54)	0.79
Hamburger (servings/wk), median	0.16	0.65	0.82	1.5	2.5	
Cases/controls	82/2,457	113/3,099	120/2,640	134/2,782	132/2,473	
Multivariable*	1.00	0.97 (0.72-1.30)	1.19 (0.88-1.60)	1.22 (0.91-1.64)	1.24 (0.91-1.70)	0.10
Multivariable (+MDM)	1.00	0.94 (0.70-1.27)	1.14 (0.85-1.54)	1.16 (0.86-1.57)	1.17 (0.85-1.61)	0.22
Multivariable (+MeIQx)	1.00	0.94 (0.69-1.26)	1.13 (0.83-1.54)	1.16 (0.85-1.59)	1.17 (0.84-1.64)	0.22
Beef, lamb, and pork as main dish (servings/wk), median	0.33	0.82	1.3	2.1	3.3	
Cases/controls	80/2,551	130/2,911	129/2,901	112/2,590	130/2,498	
Multivariable*	1.00	1.28 (0.96-1.71)	1.20 (0.89-1.61)	1.12 (0.82-1.53)	1.26 (0.92-1.74)	0.49
Multivariable (+MDM)	1.00	1.23 (0.92-1.65)	1.14 (0.85-1.54)	1.06 (0.77-1.45)	1.17 (0.85-1.62)	0.81
Multivariable (+MeIQx)	1.00	1.23 (0.91-1.65)	1.14 (0.84-1.55)	1.05 (0.76-1.46)	1.18 (0.84-1/65)	0.81
Processed meat (servings/wk), median	0.16	0.65	1.3	2.4	4.5	
Cases/controls	77/2,681	88/2,275	140/3,115	126/2,716	150/2,664	
Multivariable*	1.00	1.22 (0.89-1.70)	1.34 (1.00-1.80)	1.36 (1.00-1.84)	1.52 (1.12-2.08)	0.02
Multivariable (+MDM)	1.00	1.20 (0.87-1.65)	1.30 (0.97-1.74)	1.31 (0.96-1.78)	1.46 (1.06-1.99)	0.04
Multivariable (+MeIQx)	1.00	1.20 (0.87-1.64)	1.30 (0.96-1.76)	1.31 (0.96-1.80)	1.47 (1.06-2.04)	0.05
Chicken + turkey (servings/wk), median	0.98	1.8	2.5	3.2	4.5	
Cases/controls	137/2,895	104/2,423	132/2,737	104/2,757	104/2,639	
Multivariable*	1.00	0.94 (0.72-1.23)	1.12 (0.87-1.44)	0.88 (0.67-1.15)	0.90 (0.69-1.19)	0.38
Multivariable (+MDM)	1.00	0.93 (0.71-1.21)	1.09 (0.85-1.41)	0.85 (0.65-1.11)	0.87 (0.66-1.15)	0.26
Multivariable (+MeIQx)	1.00	0.94 (0.72-1.23)	1.13 (0.88-1.45)	0.89 (0.68-1.16)	0.92 (0.70-1.21)	0.46

*Models adjusted for age (in 5-year categories), family history of colorectal cancer (first-degree relative), reason for endoscopy (screening versus others), negative endoscopy before 1996 (yes versus no), physical activity (metabolic equivalent-hours/wk in quintiles), smoking status (never smokers, past smokers who quit ≤10 years ago, past smokers who quit >10 years ago, current smokers), race, aspirin use (<2/wk versus ≥2/wk), total energy intake (in quintiles), and calcium and folate intake (in quintiles).

(fourth versus lowest quintile: OR, 1.50; 95% CI, 1.04-2.16; highest versus lowest quintile: OR, 1.37; 95% CI, 0.92-2.05) and no associations between any of the three heterocyclic amines and adenoma were found.

We also examined whether the observed associations between MDM and adenoma could be explained by the PHM (for information on how PHM was calculated, see Materials and Methods). PHM was moderately correlated with MDM (r = 0.73), PhIP (r = 0.62), and DiMeIQx (r = 0.58) but was almost perfectly correlated with MeIQx (r = 0.96). PHM itself was associated with increased risk of adenoma (highest versus lowest quintile: multivariable OR, 1.30; 95% CI, 1.01-1.69; P_{trend} = 0.08). However, when we included both MDM and PHM in one model, there was no association between PHM and risk of adenoma and associations between MDM and adenoma were attenuated but remained similar (fourth versus lowest quintile: PHM, OR, 0.97; 95% CI, 0.68-1.37; MDM, OR, 1.38; 95% CI, 0.98-1.94; highest versus lowest quintile: PHM, OR, 1.09; 95% CI, 0.75-1.58; MDM, OR, 1.26; 95% CI, 0.87-1.82).

Adding intakes of total fat, animal protein, total iron, heme iron, vitamin C, vitamin D, total fiber, and total carotene as well as multivitamin and vitamin E supplementation separately to the models attenuated associations between heterocyclic amines and MDM and adenoma slightly, but results remained similar (data not shown).

Because large adenomas may have a higher probability than small adenomas to develop into colon cancers (6), we also investigated associations for small (<1 cm) and large (≥1 cm) adenomas separately. Risk factors that are involved in the initial formation of an adenoma may differ from those factors that cause progression of an adenoma (6, 27). Therefore, we restricted participants with small adenoma to those with negative endoscopies before 1996 to examine associations between incident small adenoma and meat mutagens. Participants with large adenoma were restricted to those without a negative endoscopy before 1996. Red meat was borderline positively associated with large adenoma (highest versus lowest quintile: OR, 1.95; 95% CI, 0.97-3.91; P_{trend} = 0.05) but not with risk of small incident adenoma (highest versus lowest quintile: OR, 0.96; 95% CI, 0.54-1.72; P_{trend} = 0.70). Processed meat intake also seemed to be associated with large adenoma, but all 95% CIs included one. Results for the three heterocyclic amines and MDM did not seem to differ considerably by adenoma size (data not shown).

Discussion

In this large prospective study, higher intake of MDM was marginally associated with increased risk of adenoma. Adjusting for total red meat or processed meat intake did not explain those associations.

Heterocyclic amines are mutagenic in animal and *in vitro* studies and are carcinogenic in animal studies (5). PhIP, the most abundant heterocyclic amine in cooked meats, is a weak mutagen, whereas MeIQx and particularly DiMeIQx are more potent mutagens (28). Therefore, we believe that summing up the individual heterocyclic amines may not be advisable because no consideration is given to the fact that individual heterocyclic amines differ with regard to mutagenic and carcinogenic potential and more weight is given to the one that is the most abundant in cooked meat (i.e., PhIP; ref. 15). MDM, on the other hand, is a measure of the total mutagenic activity found in cooked meats (http://www.charred.cancer.gov). Moreover, MDM integrates mutagenic activity from heterocyclic amines and from other yet unidentified compounds found in cooked meats (15). We therefore believe that MDM in cooked meats may be a better measure to assess a possible overall effect of cooking methods on disease risk in epidemiologic studies than measuring total heterocyclic amine in cooked meats (15).

When we included both MDM and PHM in one model, positive associations between MDM and adenoma were attenuated but remained similar. These results suggest that mutagens other than heterocyclic amines may also play a role in explaining the observed associations between MDM and adenoma. Given the collinearity between these two measures, we could not examine this hypothesis in more detail.

Epidemiologic data on the association between heterocyclic amine intake (8, 15-17) and MDM (8, 15) and risk of colorectal cancers or adenomas are sparse, and to our knowledge, no prospective data are available. In a Swedish population-based case-control study (17) with 352 colon cancer and 249 rectal cancer cases, no evidence for a positive association between higher intake of PhIP, MeIQx, and DiMeIQx and total heterocyclic amine and risk of colon or rectal cancer was observed. In fact, controls seemed to have somewhat higher total heterocyclic amine intake than cases. However, four colon cancer cases but none of the controls had heterocyclic amine intakes above 1,900 ng/d, suggesting the possibility of an increased risk at the very high end of heterocyclic amine intake (17). In addition, MDM was not assessed in that study. The Swedish heterocyclic amine database is not directly comparable with the Charred database (http://www.charred.cancer.gov) because meats were cooked to different level of doneness (29).

In contrast, in a case-control study from the United States, participants in the highest compared with the lowest quartile of MeIQx intake had an ~4-fold increased risk of developing colorectal cancers (P_{trend} < 0.05; ref. 16). In another U.S. case-control study, higher DiMeIQx intake was associated with significantly higher risk of colon cancer and higher MDM was weakly associated with higher risk (8). In the case-control study that examined the association between heterocyclic amine intake and MDM and risk of colorectal adenoma (15), positive associations between higher intake of heterocyclic amines and risk of colorectal adenoma were seen for DiMeIQx, PhIP, and MDM. After adjusting for red meat, positive associations remained statistically significant for MeIQx and MDM. However, positive associations between red meat intake and adenoma disappeared after adding either heterocyclic amine or MDM to the models (15).

For case-control studies that assess exposure after diagnosis, recall bias cannot be excluded. Therefore, the prospective design can be considered as one of the strengths of this study. All exposure data used in this analysis were collected before diagnosis of the colon adenoma and should not have been biased by the outcome. Another strength is the use of several FFQs, which enhanced our estimate of long-term dietary intake (22). Our detailed FFQs also enabled us to examine possible confounding with several nutrients and dietary factors.

However, our study also has limitations. Heterocyclic amine and MDM intake were based on a limited set of cooking method questions; however, the cooking method questions included in the 1996 questionnaire were based on the results of a detailed pilot study, which determined the group of cooking method questions that would best predict heterocyclic amine intake in this specific cohort (26). Secondly, we cannot exclude the possibility of misclassification of exposure to heterocyclic amine due to certain factors, such as frequency of flipping the meat during the cooking process or meat thickness, which were not captured in this questionnaire. Thirdly, information on cooking methods were only obtained in 1996, so repeated measurements were not available. Finally, the heterocyclic amines are not carcinogenic by themselves but need to be metabolized by activity enzymes, such as cytochrome *P*450 1A2, *N*-acetyltransferase 1, *N*-acetyltransferase 2, and sulfotransferases (30-33). Epidemiologic studies that have investigated possible interactions between genetic polymorphisms of some of these metabolic enzymes and cooking methods of

meats in relation to risk of colorectal cancer have yielded inconsistent results (9). We did not study interactions between heterocyclic amine intake and genetic polymorphisms of metabolic enzymes here.

In conclusion, higher consumption of mutagens from meats cooked at higher temperature and longer duration may be associated with higher risk of distal colon adenoma independent of overall meat intake. Because mutagens other than heterocyclic amines also contribute to MDM, our results suggest that mutagens other than heterocyclic amines in cooked meats may also play a role in increasing the risk of distal adenoma.

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